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The distribution and ultrastructure of the Kurloff cell in the guinea-pig

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INTRODUCTION

The existence of an unusual mononuclear cell, containing a single large inclusion body, in the peripheral blood of the guinea-pig has been known for many years. Increased numbers of these cells, called Kurloff cells after their discoverer (Kurloff, 1889), are present in the peripheral blood during pregnancy and after oestrogen treatment in male and female animals (Ledingham, 1940), but relatively few are present in immature, non-pregnant, and non-oestrogen-treated animals.

Histochemical studies on the Kurloff cell carried out by Pearse (1949) indicated that the inclusion body contained a mucoprotein. Marshall & Swettenham (1959) demonstrated that sulphated mucopolysaccharide was in addition present in the inclusion body, and they concluded that the inclusion material was a mucoprotein-sulphated mucopolysaccharide complex. A recent chemical analysis of the inclusion material confirmed that it consists of a protein-polysaccharide, chemically similar to chondroitin-4-sulphate, associated with a glycoprotein (Dean & Muir, 1970).

Despite the knowledge that a marked increase in the number of circulating Kurloff cells takes place during pregnancy, the function of this cell has remained speculative. However, recent studies using immunofluorescence have shown that during pregnancy circulating Kurloff cells release their inclusion material within the trophoblast (Marshall, Swettenham, Vernon-Roberts & Revell, 1970). Moreover, it has been demonstrated that purified Kurloff cell protein-polysaccharide prevents immune damage to target cells by destroying sensitized macrophages *in vitro* (Warren, Vernon-Roberts, Revell & Marshall, 1971).

The purpose of the present study is to give an account of the distribution of the Kurloff cell within various organs and to describe the ultrastructural appearances of the cell. There has been no previous systematic description of the distribution of the Kurloff cell, and our ultrastructural observations differ in several important respects from previous electron microscopical studies, which have been limited to the thymus (Bimes, Izard & Guilhem, 1963) and the spleen (Berendson & Telford, 1967).

MATERIALS AND METHODS

Male and female guinea-pigs of the Dunkin–Hartley strain (350–500 g body weight) were used throughout. Ten pregnant female animals were sacrificed during the second half of the gestation period. Ten non-pregnant females and ten males were injected subcutaneously with oestradiol benzoate (1·0 mg dissolved in 0·5 ml

arachis oil) 14 and 7 days before sacrifice, and six female animals were given a single dose of oestrogen 6 days before sacrifice. For light microscopy, tissues were fixed in 4 % formaldehyde-saline, embedded in wax, and sectioned at 5 μm. Sections were stained using the periodic acid–Schiff, alcian blue (pH 0·2) and toluidine blue methods described by Marshall & Swettenham (1959), and by haematoxylin and eosin. Blood films and spleen imprints from oestrogen-treated animals were air dried, fixed in 70 % methyl alcohol and stained with Giemsa. To demonstrate acid phosphatase, cryostat sections of unfixed spleen and kidney were stained by the Gomori technique.

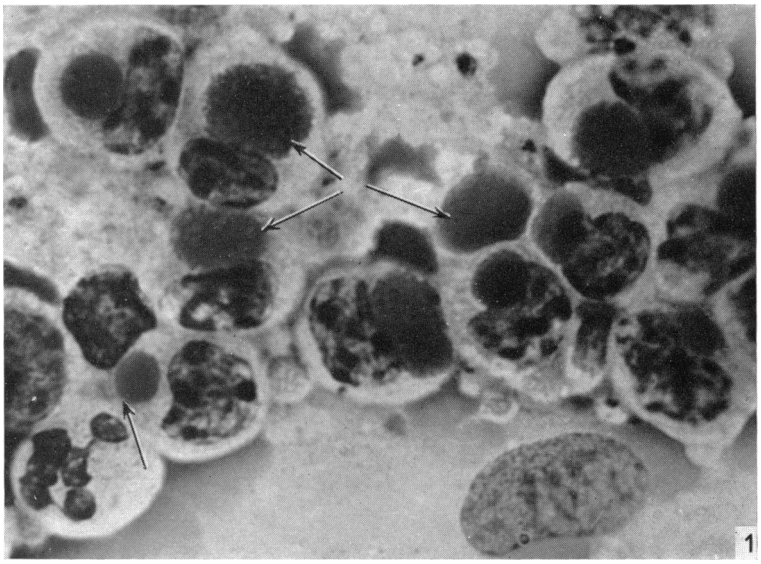
For electron microscopy, tissues were fixed in 2·5 % phosphate-buffered glutaraldehyde (pH 7·4) at 4 °C, then treated with Palade's 1 % osmium tetroxide and embedded in Araldite. Sections were cut using an LKB Ultrotome 1, stained with uranyl acetate, mounted on unsupported copper grids and examined in an A.E.I. EM 6B electron microscope. For acid phosphatase demonstration, equal numbers of millimetre blocks of spleen were fixed in glutaraldehyde or 2·5 % formaldehyde (both fixatives buffered to pH 7·4 with M/15 phosphate), washed in buffer and incubated in Gomori's acid phosphatase substrate. Blocks were re-washed in buffer. Post-fixation with osmium tetroxide and embedding in Araldite was carried out as described above.

RESULTS

Light microscopy

The Kurloff cell (Fig. 1) is ovoid in shape and the long axis of the cell is between 8 and 12 μm in length. The inclusion occupies most of the cytoplasm and is rounded or ovoid in shape; it measures 1–8 μm in diameter. The nucleus is round or indented. The rim of the cytoplasm surrounding fully formed large inclusion bodies is generally so thin that it is difficult to distinguish with certainty by light microscopy. In sections stained by haematoxylin and eosin the Kurloff cell can be remarkably difficult to distinguish since the inclusion body has the size and staining characteristics of a red blood corpuscle, and has been confused with the latter in some publications. However, the Kurloff cell may be identified with ease in sections stained by other methods. The inclusion body stains magenta using the periodic acid–Schiff technique, blue-green using alcian blue at pH 0·2, and metachromatically when toluidine blue is used. The cells are also easily recognized by phase-contrast microscopy in unstained preparations.

In the thymus (Fig. 2), small numbers of Kurloff cells were occasionally seen in the medulla adjacent to the cortico-medullary junction. Large and small clumps of Kurloff cells were seen in the cortex at the cortico-medullary junction, in some instances forming an almost confluent band of cells demarcating the junction. A small proportion of the cells in this region possessed inclusions varying in size from being just discernible to a maximum of about 8 μm diameter. Mitoses were not observed in cells containing the characteristic inclusion body. Kurloff cells were scattered in small numbers throughout the cortex, and at its periphery they formed abundant clumps and also distended lymphatic channels. Lymphatics and venous channels in the thymic fat contained many Kurloff cells, whereas the arterial circulation contained relatively few.



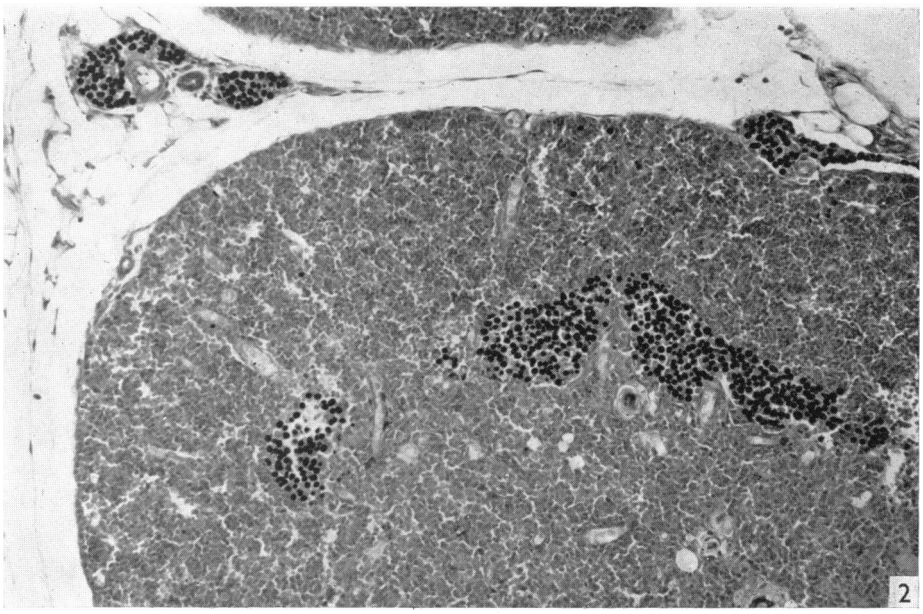
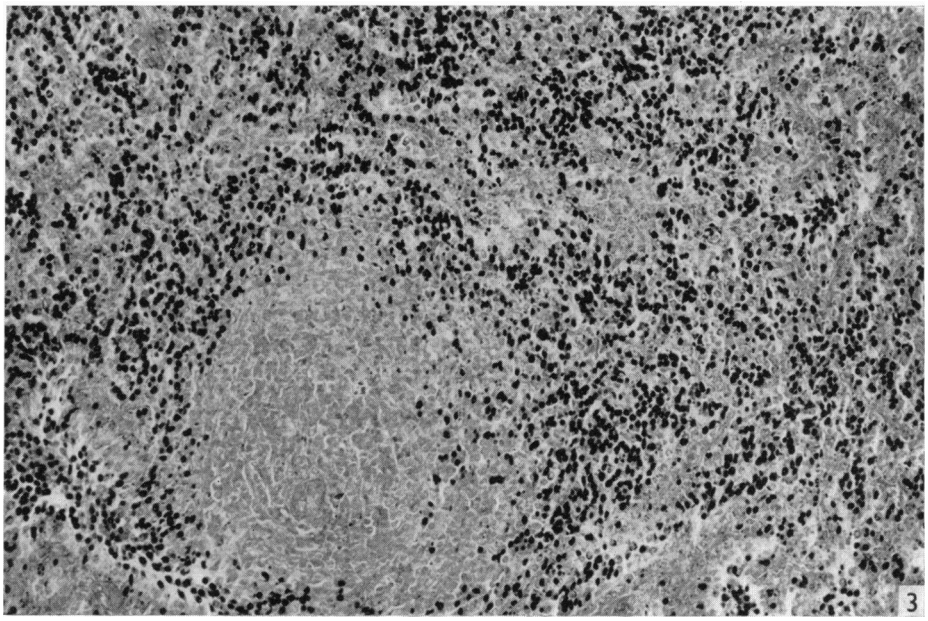


Fig. 1. Spleen imprint showing Kurloff cells with inclusion bodies (arrows) of various sizes. ×1250. Giemsa.

Fig. 2. Thymus showing clumps of Kurloff cells at cortico-medullary junction, scattered single cells in the cortex, and numerous Kurloff cells in lymphatics and venules (Kurloff inclusions appear black in the photomicrograph). ×150. P.A.S.



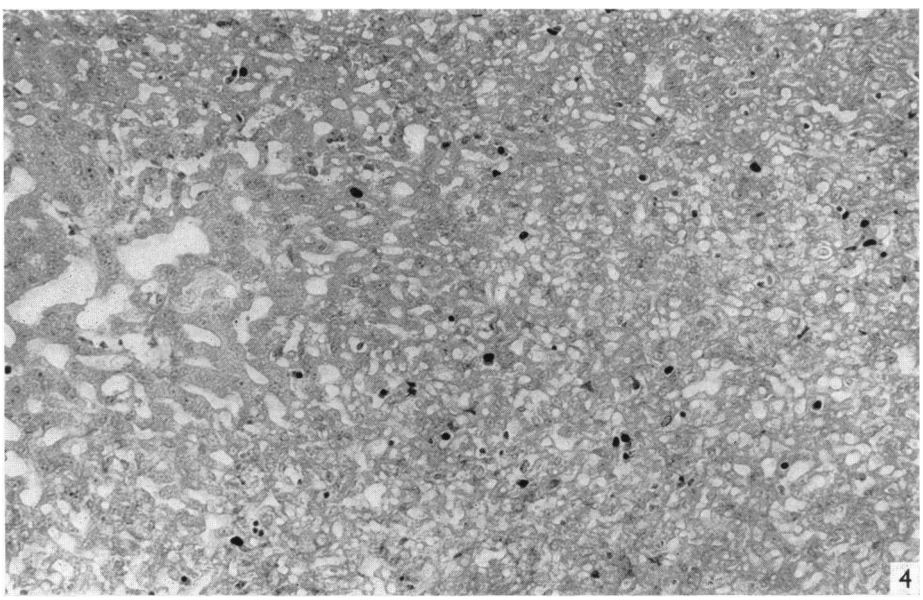


Fig. 3. Spleen showing abundant Kurloff cells in red pulp and their absence from white pulp. ×150. P.A.S.

Fig. 4. Placenta showing many Kurloff cells in the vascular channels of the labyrinth and their absence from the plasmodial spongy zone. ×150. P.A.S.

In the spleen (Fig. 3), Kurloff cells were not seen in the lymphoid tissue of the white pulp, whereas there were large numbers in the red pulp. The majority of Kurloff cells were situated in the splenic cords, and much smaller numbers lay free within the venous sinuses. In the splenic cords, many cells possessed inclusions varying in size from those which were just discernible to those which considerably distended the cell. Mitoses were not observed in cells possessing the characteristic Kurloff inclusion. Abundant Kurloff cells were present in the splenic veins, but relatively few in the splenic artery.

In the vertebral and femoral bone marrow, Kurloff cells were seen scattered as single cells and in clumps in random fashion among the haemopoietic cells in the stroma, particularly in the metaphyseal region. A few single Kurloff cells were constantly present within the sinusoids.

In lymph nodes, small numbers of scattered isolated Kurloff cells were occasionally seen in the subcapsular sinusoids and within the medullary sinuses near the hilum. No Kurloff cells were seen in lymphoid follicles or in parafollicular areas.

In the placenta (Fig. 4), considerable numbers of Kurloff cells were seen in vascular channels. They were more numerous in the placental labyrinth than in the plasmodial spongy zone of syncytiotrophoblast. Cells were occasionally surrounded by a haze of very small discrete droplets of material having similar staining characteristics to the Kurloff inclusion body. A layer of similar material was also frequently seen lying on the luminal surface of vascular channels in the placental labyrinth.

In the lung, large numbers of Kurloff cells were constantly seen within alveolar capillaries. Occasional cells appeared to be lying on the alveolar walls within the alveoli, but extravascular Kurloff cells were not found in lungs examined by the electron microscope.

In liver, intestine, kidney, adrenals, skin and striated muscle, Kurloff cells were present solely within vascular channels.

There was no discernible difference in the numbers of Kurloff cells in the thymus or spleen of pregnant animals when compared with those of males and females which had received two doses of oestrogen before sacrifice. The animals which had received a single injection of oestrogen six days before sacrifice possessed fewer Kurloff cells, and a greater proportion of them exhibited inclusion bodies below 8 μm diameter.

Sections of spleen stained for the presence of acid phosphatase did not reveal the presence of this enzyme within Kurloff cells, whereas macrophages and neutrophil polymorphs exhibited a normal positive reaction.

Electron microscopy

Variations in the size and appearance of the inclusion body constitute the principal morphological difference between individual Kurloff cells, and there do not appear to be any specific features which distinguish Kurloff cells seen in various sites.

The cell (Fig. 5) is limited by a plasma membrane of about 8 nm in thickness. The outline of the cell is generally smooth, but a small number of microvilli may be seen, and occasional cleft-like invaginations of the plasma membrane, often quite deep, are observed. Evidence of pinocytosis, in the form of pinocytotic vesicles and invaginations, is frequent.

The nucleus may be round, ellipsoid or indented, and is bounded by a nuclear

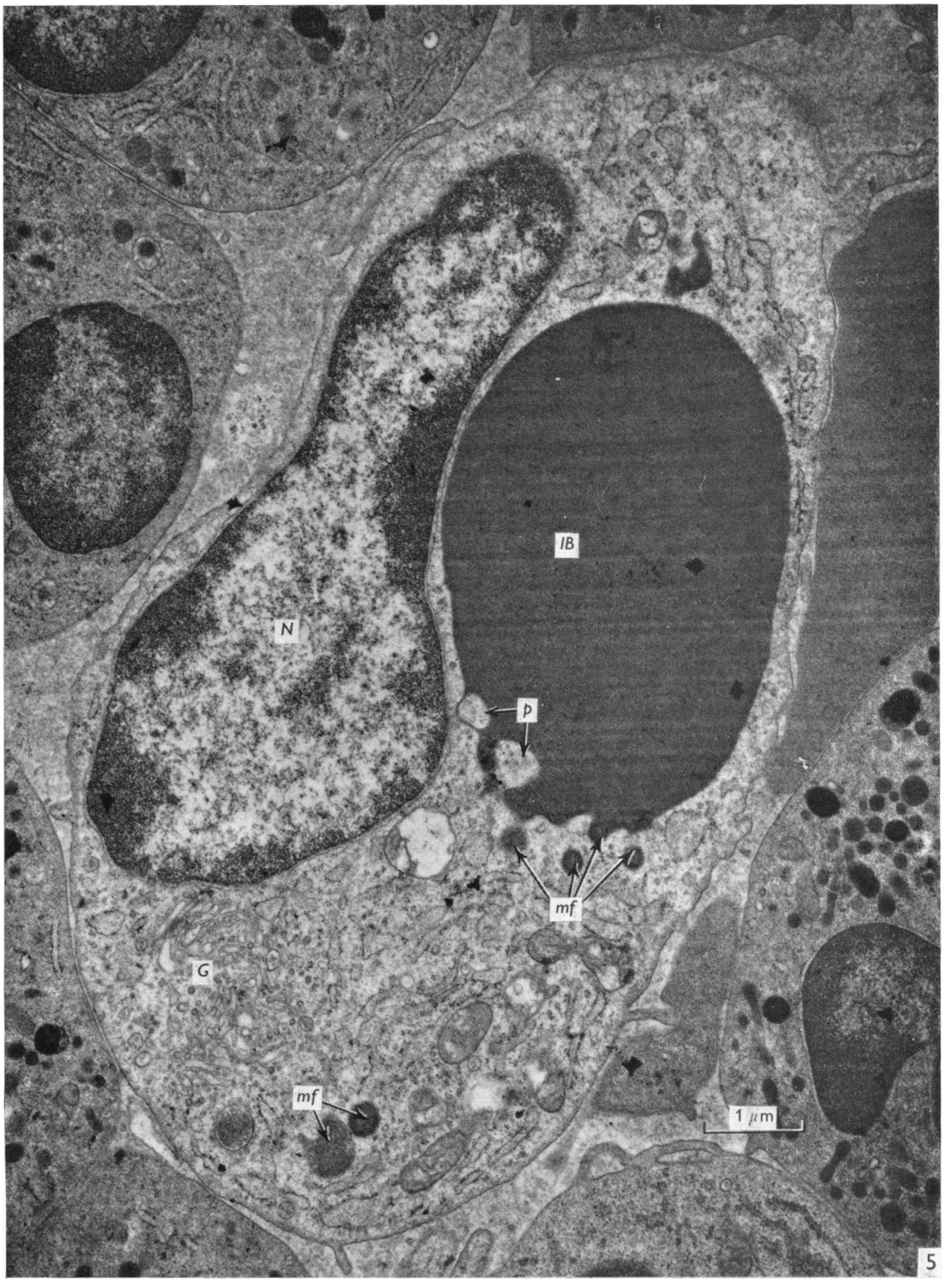
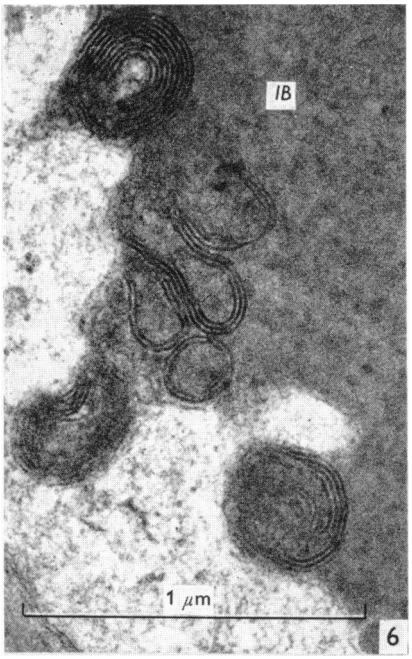
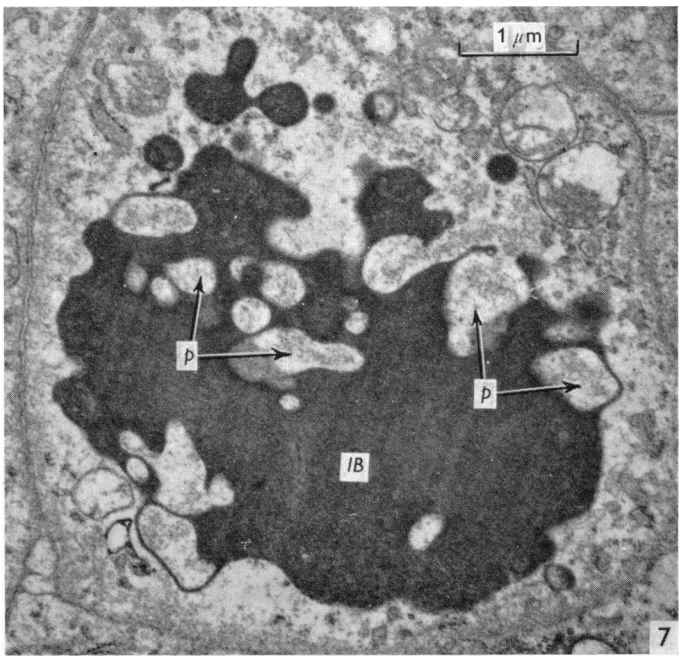


Fig. 5. Kurloff cell in bone marrow showing inclusion body (*IB*) and indented nucleus (*N*). There are myelin figure formations (*mf*) situated within the periphery of the inclusion, in the cytoplasm adjacent to the inclusion, and elsewhere in the cell. Villous projections of cytoplasm (*p*) extend into the inclusion. There is a prominent Golgi complex (*G*).





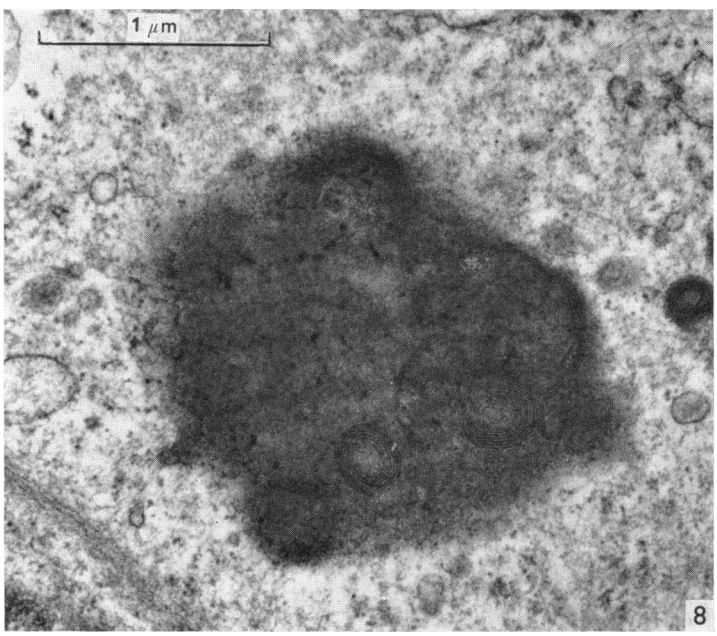


Fig. 6. Part of edge of inclusion body (*IB*) of Kurloff cell showing myelin figures situated in close association with the limiting membrane of the inclusion body.

Fig. 7. Appearance of inclusion body (*IB*) of Kurloff cell sectioned near edge, showing apparent islands (*p*) of cytoplasm within the matrix of the inclusion.

Fig. 8. Tangential section through edge of inclusion body showing some myelin figures apparently separated from the limiting membrane.

envelope containing pores. The nuclear chromatin is usually marginated in irregular clumps (Fig. 5). Generally, one nucleolus is observed.

The most striking feature of the cells is the inclusion body (Fig. 5). It is always single, round or oval in outline, and moderately and homogeneously electron-dense. It is bounded by a unit membrane, about 8 nm thick, which contains no pores. Most inclusion bodies have abundant myelin figures situated within their periphery (Figs. 5, 6 and 8). In places, a combination of myelin figures may have a complex arrangement at the edge of the inclusion body (Fig. 6). Membrane-bound projections of the inclusion body are seen to extend into the surrounding cytoplasm and contain similar homogeneous electron-dense material or myelin figure formations (Fig. 7). Examination of serial sections confirms that these myelin figures are always situated close to the membrane surrounding the inclusion body, although they may appear to be within the matrix of the inclusion when sectioned tangentially (Fig. 8). Myelin figures having no connexion with the inclusion body are occasionally seen in the cytoplasm (Fig. 5). Villous projections of cytoplasm extend into the inclusion body and, when the edge of the body is sectioned, can give the appearance of circumscribed islands of pale granular material (Fig. 7).

The centrosome and the Golgi apparatus are usually situated between the nucleus and the inclusion body or in the angle between these two structures (Fig. 5). The Golgi apparatus occupies a variable area, and usually consists of 2–4 groups of cisternae and of microvesicles (Fig. 9). Microvesicles, with an average diameter of 50 nm and containing material of variable electron density, may be seen in relation to dilated segments of Golgi saccules. Some small electron-lucent microvesicles are seen close to the cisternae. Larger smooth-membraned microvesicles have a diameter ranging from 80 to 250 nm and contain material of variable density which may appear as a condensed core separated from the membrane by a clear space of variable width. They do not appear to be related to dilated segments of Golgi cisternae but tend to be grouped between the lamellar stacks of cisternae. Multivesicular bodies sometimes occur in the Golgi region.

Small coated microvesicles are occasionally seen in the cytoplasm, and are constantly observed in the Golgi region, where they may be closely associated with the cisternae (Fig. 9).

Bundles of microfilaments are present in the cytoplasm of the perinuclear area and around the inclusion body.

Many ribosomes and polysomes are found free in the cytoplasm. Ribosomes are also attached to the external nuclear membrane, and to the abundant rough endoplasmic reticulum (RER), which consists of arrays of cisternae containing material of moderate electron density. The RER is usually located in circumferential fashion around the periphery of the cell cytoplasm. A constant feature is the presence in it of a small number of focal dilatations of the cisternae containing granular material of moderate electron density. Smooth endoplasmic reticulum is not seen.

Mitochondria are present in greater numbers than elsewhere in the cytoplasm adjacent to the inclusion body. Many of the mitochondria situated in this region and a few of those elsewhere are swollen, vacuolated, and exhibit loss of cristae (Fig. 5).

There is no evidence that these cells normally undertake phagocytic activity since they do not contain lysosomes or phagosomes. The inclusion body, myelin figures,

Golgi apparatus, microvesicles and multivesicular bodies do not exhibit an acid-phosphatase reaction product when the appropriate staining technique is used.

Electron microscopy confirms our light-microscopical observations that Kurloff cells are freely admixed with other cells in the stroma of thymic cortex and bone marrow, and within the splenic cords. The Kurloff cells do not bear any particular relation to, or arrangement with, the other component cells of these tissues. In these

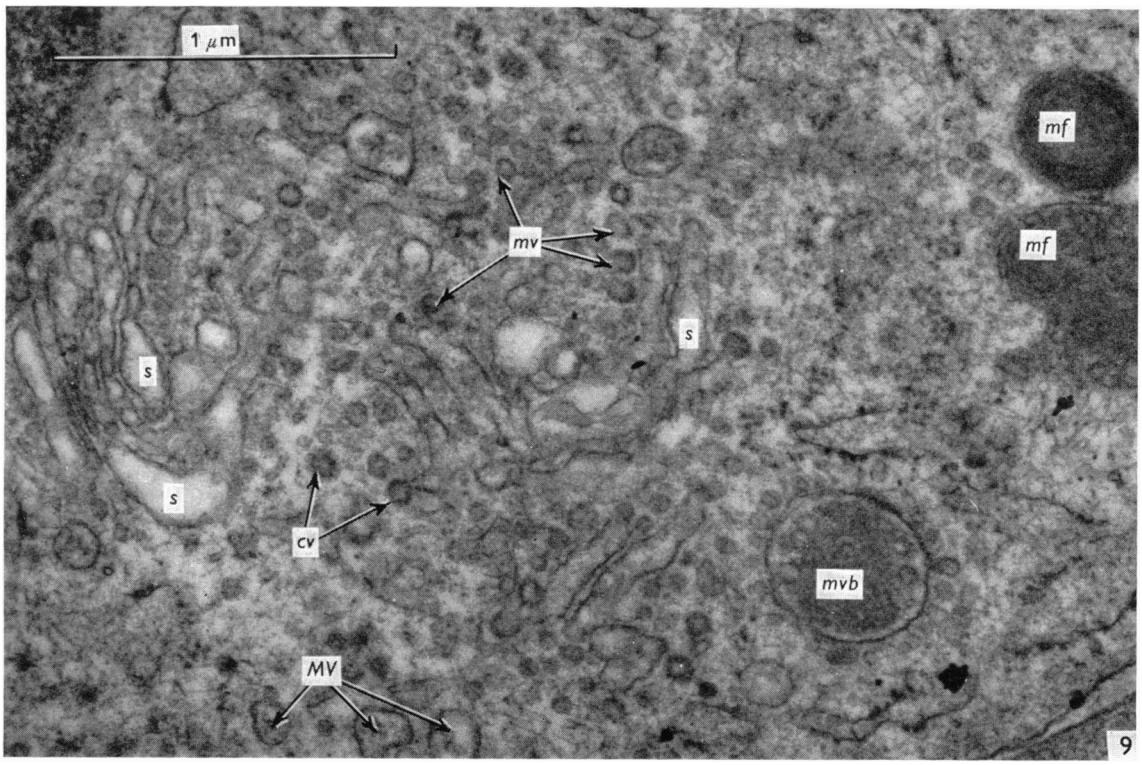


Fig. 9. Higher magnification of the Golgi region of the cell shown in Fig. 5. Shows spatial relationship between the dilated saccules (*s*) of the Golgi complex, small coated microvesicles (*cv*), small microvesicles (*mv*) and large dense-core microvesicles (*MV*). A multivesicular body (*mvb*) is present. Two myelin figures (*mf*) are shown, one containing some microvesicles.

sites, and not elsewhere, a proportion of the Kurloff cells contain inclusion bodies varying in size from very small to the large inclusions seen in circulating Kurloff cells. Cells containing more than one inclusion body are not seen. This is in contrast to the appearance by light microscopy when cells containing two or three very small inclusions which have similar staining characteristics to the Kurloff inclusion body are occasionally observed. Kurloff cells in vascular channels tend to have fewer microvilli than those in the stromal tissues of thymus, spleen and bone marrow, but do not differ in any other respect.

DISCUSSION

We have found that, in pregnant and oestrogen-treated male and female guinea-pigs, clumps of Kurloff cells containing inclusion bodies of variable size are present in large numbers in the stromal tissues of the thymus and bone marrow, and in the pulp cords of the spleen. Moreover, the veins and lymphatics draining these tissues contain strikingly large numbers of Kurloff cells relative to the numbers in the arterial blood supply. These findings suggest that the Kurloff cell may be produced at these sites. The morphology and identity of the precursor cell is unknown, and it is possible that the precursor proliferates elsewhere and migrates to these sites, where the inclusion body is formed. Very few Kurloff cells are observed within lymph nodes, and those which are present are in the sinuses and blood vessels.

During pregnancy and after oestrogen treatment, Kurloff cells circulate in large numbers in the blood. However, the numbers present in the placenta appear to be greater than can be accounted for by the vascularity of this organ. In the placenta, Kurloff cells were occasionally seen surrounded by a haze of very small discrete droplets of material having similar staining characteristics to the inclusion body, and similar material was frequently seen lying on the luminal surface of vascular channels in the placental labyrinth. These findings are in accord with those of Marshall *et al.* (1970) who have shown by immunofluorescence that the Kurloff cell releases its inclusion material in this region, and who have postulated that the cell may play some part in protecting the trophoblast against immunological damage by maternal cells.

The nature of the inclusion body has been a source of debate in the past. Authors have variously described it as being phagocytosed material, an ingested red blood cell, an intracytoplasmic parasite, and remnants of degenerate nuclei. Recent studies by Dean & Muir (1970) have established beyond doubt that the inclusion body is composed of a glycoprotein associated with a unique protein-polysaccharide similar in structure to chondroitin-4-sulphate. The question still remains as to whether the inclusion body represents the product of a secretory process by the cell itself, or represents the outcome of phagocytic uptake of material produced by some other cell. In this latter connexion, the Kurloff cell does not contain lysosomes or phagosomes, and neither the inclusion body nor any of the intracytoplasmic components of the Kurloff cell exhibits the positive acid-phosphatase staining reaction which would be expected in a cell carrying out phagocytic activity. Moreover, the injection of extracted Kurloff inclusion material into guinea-pigs does not result in the formation of increased numbers of Kurloff cells (Marshall & Swettenham, 1959). These findings are at variance with those of Izard, Bimes & Guilhem (1964), who examined the thymus and suggested a lysosomal origin for the Kurloff inclusion body; this suggestion was based on the ultrastructural presence of acid phosphatase, which was not, however, present in the light microscopical preparations of these authors.

The occasional presence of coated microvesicles in the cytoplasm and their constant presence in the Golgi region suggest the possible uptake of material from the extracellular fluid and its transport to the Golgi region. It is therefore not possible completely to exclude the endocytosis of extracellular matter, probably protein, from playing some part in the formation of inclusion body material.

The presence within Kurloff cells of certain intracytoplasmic organelles, revealed by the electron microscope, is evidence in favour of the cell itself actively secreting the constituent materials of the inclusion body. The cell is equipped with those organelles which are normally associated with protein synthesis, namely, ribosomes and polyribosomes, abundant rough endoplasmic reticulum and a prominent Golgi complex. The presence of a prominent Golgi complex also favours the ability of the cell to synthesize sulphated mucopolysaccharide, since it has been shown autoradiographically that chondrocytes localize ^{35}S to the Golgi complex (Godman & Lane, 1964; Peterson & Leblond, 1964). It has also been shown that Kurloff cells actively take up injected ^{35}S (Marshall *et al.* 1970) but the exact intracellular localization of the isotope has not yet been established.

We have observed occasional focal dilatations of the cisternae of the rough endoplasmic reticulum, but we have not been able to confirm the presence of extensive exceptionally dilated endoplasmic reticulum with ill-defined membranes which Berendson & Telford (1967) considered to be characteristic of the Kurloff cells of the spleen. However, in agreement with these authors we have commonly observed poorly preserved mitochondria adjacent to the inclusion body in cells in which the mitochondria situated elsewhere exhibit a normal appearance. Berendson & Telford (1967) noted the presence of abundant peripherally situated myelin figures within the inclusion body, and also reported the presence of centrally situated myelin figures within small inclusions. Our examinations of serial electron microscopical sections have revealed that myelin figure formations are always situated in close approximation to the membrane surrounding the inclusion despite sometimes appearing to be situated within the matrix of the inclusion when the body is sectioned tangentially towards its edge. The question arises as to why myelin figure formation is commonly observed at the periphery of the Kurloff inclusion body. One possibility is that lipophilic mucoprotein contained within the inclusion body may interact with the membrane surrounding the inclusion or with surrounding cytoplasm. In this connexion, lamellated bodies of similar structure to myelin figures have been produced *in vitro* by adding certain complex lipids to water (Fawcett & Ito, 1958).

The presence of myelin figures within the cytoplasm adjacent to the inclusion body but not connected to it may be related in some way to the presence of the poorly preserved mitochondria commonly observed in this region of the cell, since Hruban, Spargo, Swift, Wissler & Kleinfeld (1963) have suggested that 'focal cytoplasmic degradation', which can result in myelin figure formation, may represent a method for the disposal of organelles when a cell changes its functional state. Thus, the association of poorly preserved mitochondria and myelin figures in cells showing no other abnormal features may characterize those Kurloff cells which have ceased secretory activity and have entered a storage phase. However, it is not possible to be certain whether the appearances of these mitochondria are the result of a degenerative process *in vivo* or of changes during processing for electron microscopy.

We are unable to confirm the presence of pale granular inclusion bodies in a proportion of the Kurloff cells of the thymus, as described by Bimes, Izard & Guilhem (1963), and we believe that these appearances arise from viewing sections through the edge of the inclusion body, where there are often large infoldings of cytoplasm.

On the basis of the distribution and ultrastructural appearances which we have described, we do not feel at this stage that the Kurloff cell can be classified as belonging to any particular cell type, such as the lymphocyte or macrophage series. Until further information is available regarding the life history and function of the cell, speculation does not seem justified.

It would appear that the Kurloff cell must have a role during pregnancy in the guinea-pig, since at this time alone there is a marked physiological increase in the number of these cells in the blood and tissues. It is perhaps surprising that the guinea-pig alone possesses Kurloff-type cells since its mode of placentation does not appear to be unique. The findings that the Kurloff cell releases its inclusion material within the trophoblast (Marshall *et al.* 1970), and that this material, when purified, prevents immunological damage to target cells *in vitro* (Warren *et al.* 1971), suggest that Kurloff cells may play a part in preventing immunological damage to the trophoblast by maternal defensive cells.

Cells having a similar morphology to the Kurloff cell have not been demonstrated in species other than the guinea-pig. However, the presence in other species of cells which may be morphologically different from the guinea-pig Kurloff cell but which are functionally identical is suggested by the extraction of protein-polysaccharide, spectroscopically and biologically identical to Kurloff protein-polysaccharide, from the thymus and spleen of pregnant humans, rats and mice (Marshall, Revell, Vernon-Roberts & Swettenham, 1970, unpublished findings).

SUMMARY

The ultrastructure and light-microscopical distribution of the Kurloff cell in pregnant and oestrogen-treated guinea-pigs have been described. The cell is characterized by the presence of a single, large, homogeneously electron-dense inclusion body, and possesses the cytoplasmic organelles suggestive of secretory activity. The periphery of the inclusion body is membrane bound and often exhibits abundant myelin figures and complex folded membranes in association with the limiting membrane. Kurloff cells are present in large numbers in the spleen, thymus and bone marrow, are rarely seen in lymph nodes, and are present in large numbers in vascular channels in the placenta.

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